ABSTRACT

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The Study of Secondary Metabolites in Explant Culture of *Trifolium pratense* L.

The basic prerequisite for a successful elicitation that is used to increase the production of secondary metabolites is, among others, finding a suitable elicitor, its concentration and optimal duration of effect of the elicitor on the plant in vitro culture, which was the main subject of this diploma thesis. The focus of our observations was the influence of 6-, 24- , 48- and 168-hour effect of nickel chloride solution (in the concentrations of 0.1 mmol, 1 mmol, 10 mmol and 100 mmol) and zinc sulphate (in the concentrations of 0.1 µmol, 1 µmol, 10 µmol a 100 µmol) on the production of flavonoids and isoflavonoids in the suspension culture of *Trifolium pratense* L. variety Tempus.

The culture was cultivated on the Gamborg nutrien medium with the addition of 2 $mg.l^{-1}$ 2,4- dichlorophenoxyacetic acid and 2 $mg.l^{-1}$ 6- benzylaminopurine at 25°C and the light period of 16 hours light/ 8 hours dark.

The maximum content of flavonoids, which was found out by the photometric determination of the Czech Pharmacopoeia 2009, was proved in the suspension culture of *Trifolium pratense* L. variety Tempus (0.406%) after 48-hour elicitation of nickel chloride solution in the concentration of 0.1 mmol, when there was a statistically significant increase in production by 782% contrary to the control culture. The highest content of flavanoids after elicitation of zinc sulphate solution (0.236 %) was achieved after 6-hour elicitation of solution in the concentration of 100 µmol, when there was a statistically significant increase in production by 419% contrary to the control culture.

The maximum content of isoflavonoids discovered by using HPLC was proved after 48-hour elicitation of the suspension culture of *Trifolium pratense* L. variety Tempus of nickel chloride solution in the concentration of 10 mmol.